THE MORPHOLOGY OF GONYOSTOMUM SEMEN FROM WOODS HOLE, MASSACHUSETTS

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The rarely observed flagellate, *Gonyostomum Semen* (Ehrenb.) Diesing,[1] was collected during July, 1934 in a plankton haul from a sphagnum swamp near Woods Hole, Massachusetts, by Miss Hannah T. Croasdale. The swamp is one of a number of sphagnum bogs found on Cape Cod and the nearby islands. Peculiar to it is a dense growth of the white cedar, *Chamæcyparis thyoides* (L.) BSP., various species of Ericaceæ, etc., which extend throughout the broad shallow margin to the edge of a pond in the center. The locality is commonly referred to by residents of Woods Hole as the Cedar Swamp. It is situated about a quarter of a mile east of the village on Nobska Road.

The waters of the pond appear brownish even in thin layers in transmitted light and are almost black in reflected light. Miss Croasdale, who made monthly pH determinations during the past four summers (1931–1934) in open water in various parts of the swamp, says that the pH remains consistently between 4.4 and 4.6. Our own determinations made during the summer of 1934 lie within this range. The bottom of the pond is covered with decomposing vegetable debris in a finely divided state, so that even when slightly agitated the water becomes opaque. The southern part of the pond, where our collections were taken, is shaded for most of the day by the dense growth of white cedar, etc.; little direct sunlight reaches this part of the lake except for a few hours at midday. Collections taken early in the morning (seven o'clock Standard Time) invariably contained so many individuals of *Gonyostomum* that the brownish water became green in the net and collecting bottle. Collections taken at noon or thereabout

[1] Gonyostomum Semen (Ehrenb.) Diesing, *Sitz.-ber. k. Akad. Wiss. zu Wien*, **52**: 332. 1865.

Monas Semen Ehrenberg, *Ber. Verh. k. preuss. Akad. Wiss. in Berlin*, **1853**: 191. 1853.

Rhaphidomonas Semen (Ehrenb.) Stein, Organismus der Infusionsthiere 3 (1). Taf. XIII, Fig. 6–12. 1878.

Since botanical nomenclature among the flagellates begins with Linnaeus' *Species Plantarum*, 1753, according to the decisions reached by the Third International Botanical Congress (1910), we must accept the earliest valid name, *Gonyostomum* Diesing, if we are to keep the group separate from the genus *Monas* Ehrenb.

brought few individuals of *Gonyostomum*, but many rotifers and trachelomonads. These observations led us to suppose that the organisms are heliophobic and that a diurnal migration takes place to and from the deep waters according to the intensity of the sunlight which strikes the surface. Lemmermann (1910), on the other hand, describes *Gonyostomum Semen* as 'photophilic.' Further observations are necessary to clear up this matter.

Collections were taken by pouring approximately five gallons of the swamp water, uncontaminated with decomposed material from the bottom, through a net of No. 20 silk bolting-cloth and straining until 200–300 cc. of the liquid remained. This quantity was poured into wide-mouthed, unstoppered bottles and taken at once to the laboratory. When placed in diffused light, the organisms lived in an apparently healthy condition for two or three weeks. By the end of this period, either the rotifers had consumed all of the individuals of the culture or other changes in the medium became so pronounced that the remaining *Gonyostomum* cells burst or encysted. In these cultures in the laboratory, division and, later, encystment of the vegetative cells were observed.

The literature is chiefly vague and non-committal concerning the details of morphology and physiology of *Gonyostomum Semen*, as likewise of other members of the Chloromonadophyceæ (Chloromonadida) with the possible exception of *Vacuolaria virescens* Cienk. (Cienkowsky, 1870; Senn, 1900; Bütschli, 1883). Perhaps this lack of information is due to the apparent rarity of the organisms, which have been reported infrequently since their first recognition. The actual localities for which we find our species previously recorded are three: a sphagnum bog near Berlin (Ehrenberg, 1853; Stein, 1878), a bog near Fölisö in Finland (Levander, 1894), and 'Sphagneten' near Seefeld and Larss in the Tyrol (Dalla Torre and Sarnthein, 1901). Pascher (1913) says of its distribution: "In stehenden Gewässern, Tümpeln und Torfsümpfen; verbreitet, doch vereinzelt." Even if others have observed the organism at other stations, Ehrenberg, Stein, and Levander appear to be the only workers who have contributed to our previous knowledge of the species.

These authors, and compilers of works on the flagellates after them, disagree on certain important details of morphology and physiology of *Gonyostomum Semen*. The flagella, for example, are described variously. Ehrenberg (p. 191) says ". . . ciliis pluribus vibrante." Diesing (1865), who obviously did not see the organism himself, remarks of the genus, "flagello ignoto." Stein mentions and figures two flagella, one projected forward and one trailing.

Levander figures only the forward-projecting flagellum and does not commit himself as to the presence of a trailing one. Similar uncertainty exists concerning the long slime-threads produced from trichocyst-like rods embedded in the cytoplasm. Lemmermann and Pascher place upon Levander all responsibility for the idea that the slime-threads become several times the length of the body of the organism. We find the following statement in Ehrenberg's original diagnosis: "Facile diffluendo ovula glandulam et spiculas bacillares tenues ostendit." It is probable, as Kent (1880) has pointed out, that Ehrenberg's phrase "ciliis pluribus" quoted above refers to the slime-threads when only partially discharged. Likewise, the literature is vague or contradictory as to the function of the triangular cavity and the nature and points of discharge of the contractile vacuoles. Cell division and encystment have not heretofore been described.

Morphology of the Vegetative Cell

In shape and size, the vegetative cells of *Gonyostomum Semen* actually vary more widely than other authors intimate. Generally speaking, the cell body (Figs. 1 and 2) is ovoid and somewhat flattened dorsoventrally, with a short, cylindrical, more or less pointed caudus at the posterior end. The dorsal surface may be ovate, obovate, ovate-lanceolate, obovate-lanceolate, or almost lanceolate or circular in outline, 1–2.5 times as long as broad in our material. In contour, the dorsal surface is convex, often with some irregularities. The ventral surface is similar, except that a shallow longitudinal furrow extends from the opening of the triangular cavity to the posterior region (Fig. 2). This furrow may be lacking in the more flattened and nearly circular types, as also in the extremely lanceolate types. In side view, the organism appears lanceolate to linear-lanceolate, with the widest part near the anterior end. Material examined an

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Explanation Plate I

Fig. 1. Vegetative cell of *Gonyostomum Semen*, ventral surface, showing the two flagella, the chromatophores, the triangular cavity, the trichocyst-like rods, and the position of the nucleus. × 944.

Fig. 2. Diagrammatic representation of side and ventral views of the vegetative cell. The ventral groove and positions of flagella are indicated. × 944.

Fig. 3. Diagrammatic drawing of a cell mounted in 1 per cent acid fuchsin, showing the slime threads. × 377.

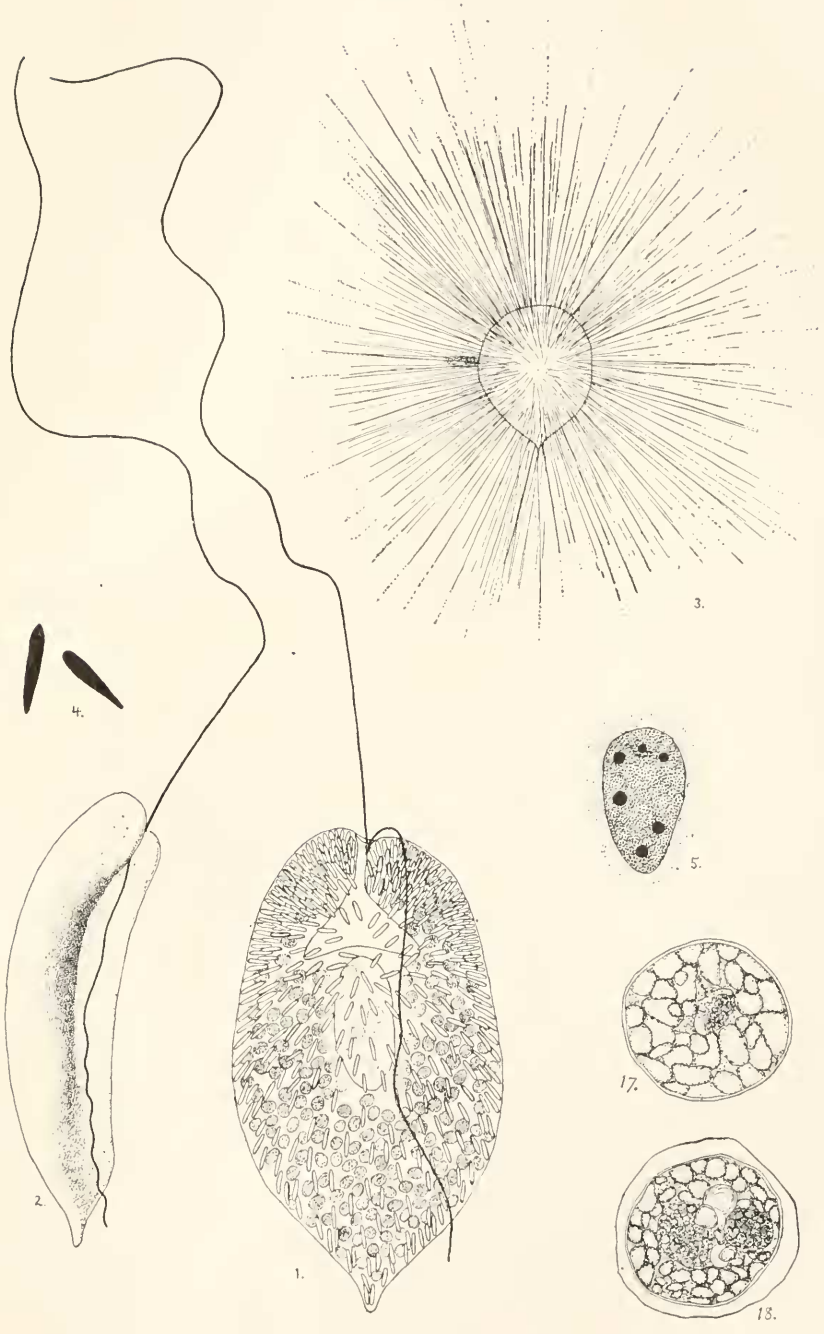
Fig. 4. Trichocyst-like rods discharged into the medium when the cell is mounted in very dilute aqueous acid fuchsin. × 2833.

Fig. 5. Nucleus as stained with cotton blue by Maneval's technique. The large endosome-like bodies and deeply-staining granular matrix are shown. × 944.

Fig. 17. Young cyst, showing the thin gelatinous sheath. × 708.

Fig. 18. Older cyst, with thick sheath, few chromatophores, and much oil. × 708.

PLATE I



hour after collection from the Cedar Swamp exhibited as a rule an abundance of the more lanceolate types (as seen in dorsal view); after a day or two of standing in the swamp water cultures in the laboratory, the organisms became more flattened and consequently broader in dorsal outline, so that almost circular cells were often observed; and the lanceolate types were indeed rare. In dorsal view, as also in ventral view, the anterior end is somewhat two-lipped, with the opening of the triangular cavity between the lips. Levander notes that the left lip (as seen in ventral view) is higher than the right lip, an observation which holds true in our material. Under certain external conditions, the caudus disappears entirely; and an organism the shape of *Vacuolaria virescens* results. Under pressure, or in viscid media such as 0.5 per cent agar or quince jelly as prepared by Turner (1917), the organisms become amœboid (Fig. 6). From the above description, it is obvious that the cell has no definitely fixed shape as has *Chlamydomonas* or *Peridinium;* the organism may be described as metabolic, with an ability to change shape in response to a change in the environment similar to that of *Euglena* or *Peranema*.

Ehrenberg's original diagnosis of *Monas Semen* describes the organism as having a length of 1/48 line (*ca.* 45 μ). Levander cites the length as 80 μ and the greatest breadth as 34 μ. Stein, and subsequently Kent, Lemmermann, and Lindau and Melchior (1926) say 44–63.5 μ. In one hundred random measurements of individuals made on various cultures during our observations, we have found that the size varies far beyond the above described limits. The largest individuals observed in our material measured 92 $\mu \times 69$ μ; the smallest 36 $\mu \times 23$ μ. Among these hundred cells, approximately three-fourths of the measurements lay between 40–80 $\mu \times 30$–60 μ. Of the entire number the average length was 62.5 μ and the average width 41.4 μ. It is probable that under environmental conditions other than those to which the cells were subjected by us the extremes of size may be greater than our measurements indicate.

The nucleus, though obscured by other cell contents in the living cell, is easily observed, when the organism bursts, as an ovoid or subglobose body about one-fourth the length of the cell. It is found (in stained preparations) in the center of the cell, though sometimes to one side of the center or definitely in the posterior region. Stein figures the nucleus as containing a single deeply-staining body (the endosome) in its center surrounded by a well-delimited vesicle containing granular chromatic material. Levander states that the nucleus contains such chromatic filamentous structures as are seen in *Peridinium*. Our material, killed in 0.5 per cent osmic acid, preserved in

2 per cent formalin solution, fixed in very dilute chromo-acetic acid, and stained in Delafield's hæmatoxylin, shows the nucleus as a well-delimited vesicular body containing several to many almost spherical, deeply-staining bodies, each 1.5 μ or less in diameter, distributed through a coarsely granular matrix. No one or two of these can be distinguished from the rest as true endosomes, such as Stein figures for *Gonyostomum Semen* and Bütschli describes in *Vacuolaria virescens*. Slides prepared by Dr. W. E. Maneval from the material fixed in osmic acid and preserved in formalin, and stained as temporary mounts [2] with cotton blue or as semi-permanent mounts with a mixture of cotton blue and acid fuchsin, demonstrate this structure even better (Fig. 5). It is not improbable, however, that the formalin produces anomalous granules in the nucleus. In material which is dried on the slide before staining, the detailed structure of the nucleus is often obscured by the overlying chromatophores and trichocyst-like rods. No evidence of a filamentous structure of the nucleus was seen. No material was sectioned and stained.

The cytoplasm, as seen in the living cell, is hyaline, finely granular, and without evident vacuolar structure. In the periphery of the cytoplasm and sometimes throughout the mass, lie the contractile vacuoles, the chromatophores, and the trichocyst-like rods. The outer layers, in the living state, exhibit no conspicuous alveolar structure as that which Senn describes and figures in *Vacuolaria virescens*. Yet, when a cell bursts or is stained without preliminary drying, as with Maneval's stains described above, the cytoplasm in which the chromatophores etc. are embedded appears less viscous and less granular than that immediately surrounding the nucleus.

At the anterior end of the organism, a duct opening to the outside leads into a cavity in the cytoplasm usually described as triangular in optical longitudinal section (the 'dreieckige Blase' of Lemmermann and of Pascher). Oltmanns (1922) describes this cavity as "von der Form eines breiten Kegels (sie erscheint im Schnitt dreiseitig)." In our material the cavities are somewhat flattened dorsoventrally and vary in shape from one-third as wide as long (in ventral view of the cell) to twice as wide as long. The function of this cavity is not as

[2] The material is mounted directly in the following solution (Maneval): 15 parts of 5 per cent phenol, 4 parts of glacial acetic acid, and 1–3 parts of a 1 per cent aqueous solution of cotton blue. For the semi-permanent mounts, a modification of Amann's lacto-phenol (Maneval) was used: 20 grams of phenol, 20 cc. of lactic acid, and 40 cc. of glycerine are dissolved in 20 cc. of water. To this 20 per cent by volume of glacial acetic acid is added. Finally, equal parts of 1 per cent aqueous cotton blue and 1 per cent aqueous acid fuchsin are added to the mixture in quantities suitable for the type of staining preferred.

yet clearly understood. Morphologically, the triangular cavities of *Gonyostomum Semen* and of *Vacuolaria flagellata* (Stokes) Senn (Stokes, 1886 and 1888; Senn, 1900) may be homologous with the so-called 'flagellar pits' of *Thaumatomastix setifera* Lauterb. and of *Vacuolaria virescens* Cienk. (Lauterborn, 1899; Cienkowsky, 1870; Bütschli, 1883; Senn, 1900). In the works on the flagellates cited at the end of this paper, one is given the impression that the contractile vacuoles discharge into this cavity.[3] Various observations of our own, insufficient at present for definite conclusions, indicate that this supposition is plausible. However, other observations indicate that the contractile vacuoles discharge through the outer cell membrane. The development of triangular cavities in dividing cells is described below.

One large contractile vacuole, said by Levander to contract about once every minute, is present in the cytoplasm somewhere in the vicinity of the triangular cavity. Levander places it "am Ende des Geisselkanals," though in our material it is more often found nearer the outer cell membrane than the cavity. Smaller contractile vacuoles were observed in some individuals scattered through the anterior cytoplasm. These appear to coalesce sooner or later and finally to empty into the large anterior vacuole. Whether the large vacuole discharges at length through the outer cell membrane or into the triangular cavity, we cannot at present be certain.

The chromatophores are small ovoid bodies of a peculiarly bright green color—the *maigrün* of German authors. As in other chloromonads, the chromatophores of *Gonyostomum Semen* become a dull blue-green in color when treated with a dilute acid, *e.g.*, 5 per cent HCl, a reaction (Pascher, Senn) indicative of the presence of an excess of xanthophyll. The chlorophyll (Pascher) is thought to be of a chemical composition somewhat different from that encountered in other flagellates and green plants. The chromatophores are arranged peripherally in the cytoplasm over the entire body of the organism with the exception of the caudus. Red-pigmented bodies (eye-spots, stigmata) are apparently absent.

No starch or starch-like substances which produce a blue color

[3] Oltmanns speaks of the function of the cavity: "Sie ist selber nicht kontraktil, steht aber mit pulsierenden Vakuolen in Verbindung, die wohl in sie einmünden. Die Sache erinnert an Euglenen und an Peridineen." Likewise, Senn remarks: "Bei anderen Formen . . . (Rhaphidomonas und Thaumatomastix) hat sich eine constant vorhandene, nach aussen offene, nicht mehr pulsierende Hauptvacuole ausgebildet, in welche sich die seitlich entstehenden Nebenvacuolen abwechselnd entleeren (Fig. 125)." The figure cited illustrates the vacuolar system of *Thaumatomastix*, redrawn from Lauterborn.

when treated with iodine were found in the cells. Oil occurred as slightly amber-colored globules distributed through the cytoplasm of cells observed shortly after collection from the Cedar Swamp. As the laboratory cultures became older, the oil droplets grew larger and more conspicuous, especially in the broader and more flattened individuals. Often one or two large irregular oil bodies comprised half the length of the cell and measured one-third to one-half as wide as long in optical section. Since these conspicuous droplets were not seen in small individuals which showed evidence of recent or future division, it is supposed that they indicate a pathological condition of the protoplasm. Similar production of excess oil under unfavorable cultural conditions was observed by Bohlin (1897) in *Chloramœba heteromorpha*. Treatment with 0.5 per cent osmic acid for a week or more at room temperature blackens the oil droplets entirely. A saturated alcoholic solution of Sudan III causes the cells to burst and allows the droplets to lie free in the medium; these droplets absorb the Sudan III readily. When organisms killed in the fumes of osmic acid are allowed to dry on the slide, the oil globules are easily discerned in the dried cells. If a drop of xylene or Canada balsam is placed on the material, the oil globules disappear. Cells fixed by other agents likewise lose the oil droplets when mounted in xylene or balsam. Levander records the finding of paramylon [4] in the cells of *Gonyostomum Semen*. He gives us no reason to suppose that he investigated the chemical nature of these bodies in the painstaking manner employed by Bütschli (1906) on the paramylon bodies of certain Euglenophyceæ.

Two flagella are present, each as long as, or longer than, the body of the organism. The one projected forward (tractellum) is attached to the inner wall of the duct leading from the triangular cavity. The insertion of the trailing flagellum (pulsellum) has not been determined; however, if it were inserted on the wall of the duct its basal portion would surely have been seen there. It is probably attached to the cell membrane near the anterior end of the ventral groove, as Stokes has recorded in *Vacuolaria flagellata*. As Levander has already noted, the organism progresses forward in a slow, spiral course, the basal portion of the tractellum projected directly in advance, moving little if at all, the apical portion spiralling so rapidly that it is invisible until the organism has come to rest. Then, when the cell is quiescent, such as following the addition of very dilute acid fuchsin (1 drop of 1 per cent aqueous acid fuchsin in 50 cc. water) to the mount, both

[4] To quote (p. 34): "An der Dorsalseite des Vorderendes sah ich öfters einige ganz minimale, ringförmige Körner, die wohl Paramylum-körner waren."

flagella can be observed. The pulsellum was never seen in its entirety and is therefore shown in Fig. 1 as shorter than the tractellum. In the dilute acid fuchsin, the vibratile portion of the tractellum lashes back and forth in slow spirals; the pulsellum often leaves the ventral groove and is projected outward or forward. The attenuated apical portion of the tractellum was seen also by carefully focusing the oil-immersion objective when dark-field illumination was employed. Since the apical parts of the flagella are so delicate, an almost perfect dark field, free from foreign particles in the mount and from scratches on the glassware, is necessary. It was found impracticable to stain the flagella, since the gelatinous threads discharged from the trichocyst-like rods obscure the flagella when a stain is applied. Since no microtome sections were made, basal granules and other structures of a kinetic apparatus remain unidentified.

No cell wall is present about the organism. The cell has, as a rule, a definite shape as has *Euglena;* only under certain marked departures from the usual conditions found in the medium, however, does the shape change as rapidly as does that of *Euglena.* The protoplast itself is delimited from the surrounding medium by a very thin plasma membrane, which does not separate from the rest of the cytoplasm when the organism is placed in concentrated salt solutions like chlorzinc-iodine (Nowopokrowsky, 1911), as does a cell wall. No blue or violet color appeared during treatment with this reagent to indicate the presence of cellulose or related carbohydrates in the membrane. When the liquid beneath the cover-slip dries slowly so that the organisms are subjected to gradually increasing pressures, the cells become amœboid. Under such pressures, a definite and distinct line (or membrane) can be seen delimiting the outer cytoplasm of each lobe. Sooner or later, if the pressure continues to be increased, the membrane breaks; and the organism bursts beyond recognition. While the membrane is still intact, the flagella move slowly or remain stationary; if more water is added to the mount (and the pressure thereby diminished), the cell regains its usual shape and the flagella resume normal activity. Amœboid cells of *Gonyostomum Semen* were found in a medium containing 0.5 per cent agar in swamp water. In such cultures, the cells lived at least 24 hours with the chromatophores and other cell structures in a healthy condition and with the trichocyst-like bodies undischarged. The lobed condition here is due probably to mechanical pressure exerted by the agar on the surfaces of the cells. Amœboid shapes were likewise assumed by cells in the process of division, as described below. The fact that *Gonyostomum Semen* exhibits such 'metaboly' as do various of the Euglenophyceæ

affords further evidence, though indirect, of the absence of a true cell wall.

Distributed radially in the peripheral cytoplasm are rod-shaped bodies (Fig. 1) characterized in the living cell by their ability to absorb large amounts of dye from very dilute aqueous solutions. Ruthenium red, Gram's iodine, acid fuchsin, methylene blue, methyl green, safranin, and Delafield's hæmatoxylin are a few of the dyes absorbed in this manner. As Levander has stated, the rods are arranged in dense 'palisade-like' formation in the anterior half of the cell, and here are most abundant in the lobes on either side of the triangular cavity. Toward the posterior end the distribution is less orderly and less abundant. The objects appear to be least numerous in the caudus, though some individuals have been observed to possess as many as ten of the rod-like bodies here.

When a very dilute solution of a dye (1 drop of 1 per cent aqueous acid fuchsin in 50 cc. water) is added to the mount, a few of the rods burst suddenly through the cell membrane and become elongated into threads of slime as long as or several times longer than the body of the organism. Others come through the cell membrane and lie free in the medium as club-shaped bodies (Fig. 4), rounded at one end and pointed at the other. Others remain half on either side of the membrane, but most remain in their original positions within the cytoplasm. In any case, the bodies quickly absorb the dissolved dye, whether they are inside or outside the cell. When more concentrated solutions of dyes (such as 1 per cent aqueous acid fuchsin) are allowed to diffuse beneath the cover-slip, the rods are discharged suddenly, and the slime-threads produced become deeply stained at once, so that the cell becomes the center of a mass of radiating gelatinous threads (Fig. 3). The threads often appear branched and *en masse* are similar to a much-branched fungus mycelium surrounding the cell, as described by Levander. When discharged in a salt solution, without the addition of a dye, the threads are difficult to see except at their bases, where they have the greatest diameter when stained. If the cells are mounted in chlor-zinc-iodine, the rods do not discharge, as Scherffel (1912) has described in *Monomastix* and *Pleuromastix*, nor do they leave the cell without elongating, as Levander has described in *Gymnodinium*.

A large percentage of the cells treated with 1 per cent solutions of the dyes mentioned above burst when the trichocyst-like bodies are suddenly discharged. It is supposed here that the membrane surrounding the protoplast is broken as the rods or filaments shoot through it. Scherffel has described and figured visible holes in the cell mem-

branes of *Monomastix*, but the rods in that organism are comparatively much larger and less numerous than those of *Gonyostomum*. Often the discharge of the bodies and consequent bursting of the membrane (or *vice versa*) can be caused by pressure. This is demonstrated when a thin film of culture medium containing the cells dries on the slide. The gradually increasing concentration of dissolved materials in the medium may be partially responsible for the bursting or discharge, but similar results are obtained when the organisms dry in distilled water.

The bursting of the cells upon the addition of various dissolved materials introduces the greatest difficulty in handling *Gonyostomum Semen*. Several types of killing and fixing agents were used on the cells with disastrous results: 1 per cent–95 per cent alcohol, $HgCl_2$ (concentrated solution in water and in 50 per cent alcohol), chromo-acetic acid (1–10 drops in 20 cc. water), Flemming's solution, and 1 per cent–3 per cent formalin in water. Inverting a slide containing a drop of the culture solution over the fumes of 1 per cent osmic acid met with the best success. The organisms also were killed satisfactorily when an equal part of 1 per cent osmic acid was added to the medium. Maneval's nuclear stain [5] proved almost as successful as a killing agent. If first killed in osmic acid or Maneval's stain, the cells could be fixed in other reagents without distortion or bursting. Difficulties similar to those encountered in fixing and staining were met with also in transferring from one culture medium to another.

When a collection is taken from the Cedar Swamp and left standing in a jar of swamp water for a day, many of the organisms fall to the bottom of the jar and become aggregated there in a gelatinous stratum. A piece of such a mass under the microscope appears to be composed of cells of the usual vegetative structure lying in a gelatinous matrix. When stained with very dilute iodine or acid fuchsin, the gelatinous material is seen to be composed of mycelium-like threads such as those about organisms from which the trichocyst-like bodies have been discharged because of a chemical stimulus. The cells, upon close inspection, contain few of the rod-like bodies. It is probable that the rods are discharged because of some change in the chemical or physical nature of the medium; the slime-threads of various individuals become

[5] A variation of the first stain described in footnote 2 of this paper: 60 parts of 5 per cent aqueous phenol, 10 parts of 30 per cent aqueous $FeCl_3$, 20 parts of glacial acetic acid, and 15 parts of 1 per cent aqueous acid fuchsin. The organisms are mounted directly in this solution. If the staining is too deep, the material may be destained to the desired color with Amann's lacto-phenol (the second solution of the same footnote without the dye added).

enmeshed; the flagella are caught in the gelatinous mass and are rendered functionless; and the organisms sink to the bottom of the jar because of their specific gravity. In the gelatinous mass the penetration of osmic acid and stains is very slow. In the case of stains, at least, the dissolved material is absorbed in large quantities by the outer part of the matrix. Senn (p. 104) gives us the impression that such gelatinous coverings are produced by many flagellates: "Gelegentliche Ausscheidung weicher Gallerte ist bei sehr vielen, besonders mit Chromatophoren versehenen Formen (Euglenaceae, Chloro- und Chrysomonadineae) haüfig. Durch ungünstige Verhältnisse (Druck, Zusätze von Reagenzien) treten aus dem Periplasten geschlangelte Gallertfaden, die durch ihre nachträgliche Verquellung die Zelle in einen losen Mantel einhüllen. Mit dieser gelegentlichen Gallertausscheidung muss auch die Bildung von Gallerthüllen durch Dauercysten in Beziehung gebracht werden." In *Euglena*, Klebs (1883) described the slime-producing bodies as being attached to the cell membrane. In *Gonyostomum Semen*, the bodies do not appear to be attached to the membrane nor do they appear to be attached to the membrane in Levander's figures of *Gymnodinium* or in Scherffel's figures of *Monomastix* and *Pleuromastix*. Whether or not these bodies in the green flagellates can be viewed as true trichocysts such as those described by Mitrophanow (1905) and Schuberg (1905) in *Paramœcium* and other Ciliata remains an open question.

Cell Division

Many of the smaller individuals found in the free-swimming and the gelatinous conditions described above, upon being treated with nuclear stains, showed the presence of two nuclei within each cell (Fig. 7). Other individuals exhibited 'swallow-tails' (Figs. 8 and 9) at the posterior ends. These peculiar features appeared in the cultures regularly during midday and the early evening. They led us to look further into the matter of cell division.

Hanging-drop cultures of swamp water were constructed and observations made almost continuously during the periods 11:00 A.M. to 1:00 P.M. and 6:30 P.M. to 8:30 P.M. Standard Time. A large number of cells were seen in various stages of division, and the entire process was watched in several individuals. The time required for complete division in the swamp water cultures was regularly 45–55 minutes. If an equal quantity of quince-seed jelly were added to the hanging-drop, the movements were slowed down and the time required for the process lengthened.

Division is longitudinal, as in other wall-less Flagellata, often

slightly oblique. The cell enlarges in transverse section, becomes more metabolic and amœboid in form, and loses the flattened shape so characteristic of the vegetative individual. Usually a lobe appears at one side of the caudus, so that two 'tails' are apparent. From each arm of the triangular cavity, as seen in ventral optical section, an extension of the cavity is sent posteriorly and laterally (Fig. 9). At the extremities of these arms, two new arms are pushed out at angles to each other into the cytoplasm (Fig. 11). These enlarge to form two new triangular cavities, each opening into the original one. Accompanying or following the formation of new cavities, a longitudinal furrow extends about the long axis of the cell body, beginning with the constriction between the 'tails' or at the anterior end in a plane bisecting the original triangular cavity longitudinally (Figs. 12–15). No matter at which end it is first evident, the furrow soon becomes conspicuous at both ends and extends rapidly to the central part of the body, ultimately constricting the mother into two daughter cells (Fig. 16). The flagella move vigorously throughout this process, and the entire body of the cell becomes contorted, exhibiting much 'metabolic' activity. New flagella appear soon after the anterior end splits in division (Fig. 14), so that the dividing individual possesses four wildly lashing flagella, seemingly hastening the separation of the cells by pulling in different directions. The new triangular cavities, as may be readily seen in Figs. 11, 12, 13, and 15, are not formed originally at the anterior apices of the constricting cells; rather, they

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Explanation of Plate II

Fig. 6. Amœboid cell body produced by pressure of the cover-slip in a gradually drying mount. × 708.

Fig. 7. Two-nucleate cell found in swamp water culture at noon. Fixed in osmic acid and stained with Delafield's hæmatoxylin. × 377.

Fig. 8. Cell in the first stages of division, with 'swallow-tails' at the posterior end. × 377.

Fig. 9. Cell with elongated and 'lobed' triangular cavity and with conspicuous 'swallow-tails.' × 472.

Fig. 10. Cell with first constriction appearing in the anterior region, instead of in the posterior as in Figs. 8 and 9. × 472.

Fig. 11. Amœboid cell in division, showing the triangular cavities of the daughter cells formed at the base of the cavity of the mother cell. × 708.

Fig. 12. The same cell constricting at the anterior end. This constriction appears to bisect the triangular cavity of the mother cell. × 708.

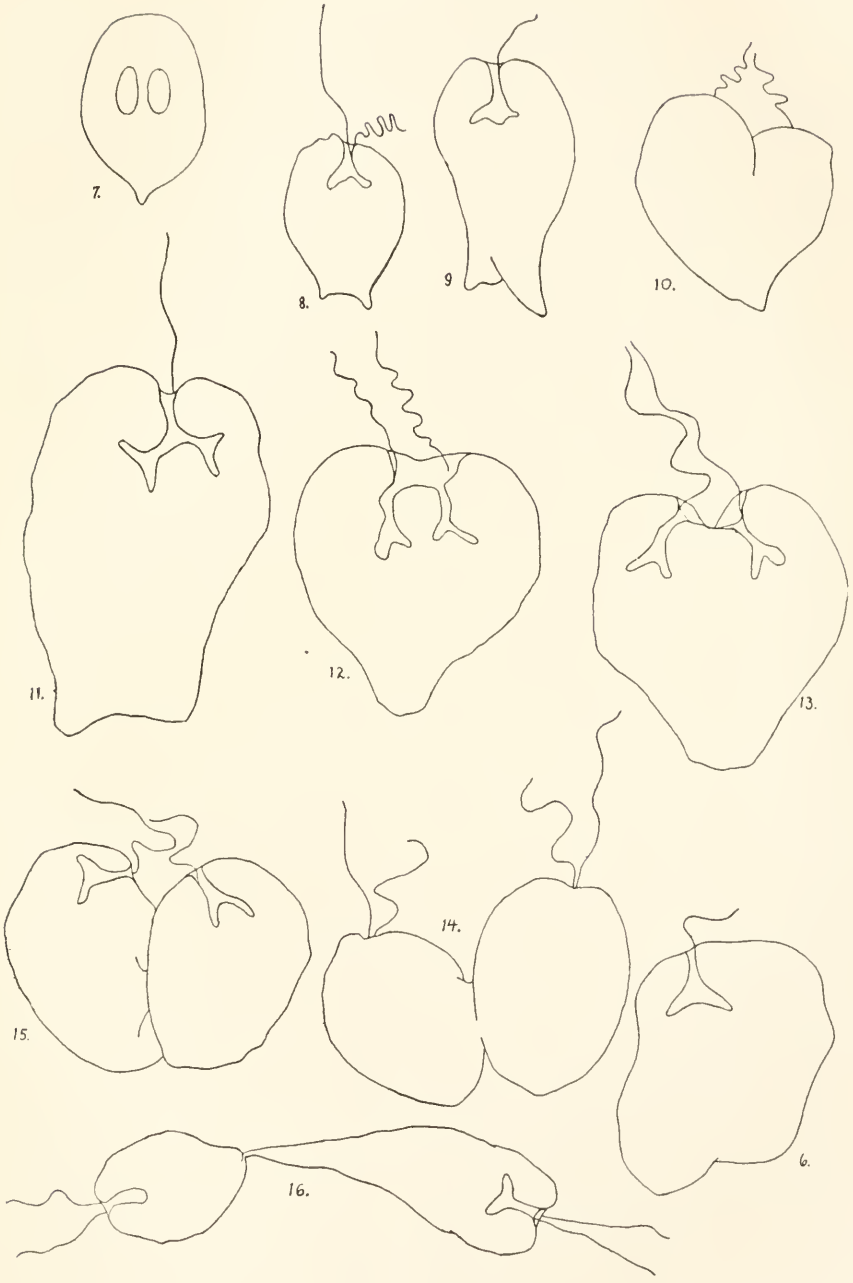
Fig. 13. A later stage in constriction of the same cell. × 708.

Fig. 14. The two daughter cells attached by only a small protoplasmic connection, each cell bearing two flagella. × 708.

Fig. 15. Same as Fig. 14, but showing the two cells with distinct triangular cavities. × 708.

Fig. 16. The same, with the daughter cells pulling apart. × 708.

PLATE II



first appear laterally, and by the splitting of the original triangular cavity and by change in shape of the daughter during the later stages of division, are finally (Fig. 16) oriented at the apex. The fate of the flagella of the mother cell and the origin of the flagella of the daughters were not noted.

As pointed out above, the dividing cells were primarily those belonging to the smaller size ranges of the population, though a few large flat cells appearing as the culture aged exhibited 'swallow-tails.' These latter types invariably contained large irregular oil bodies and possibly were pathological, for none were observed to proceed further in division. Such observations, if simulated by future studies in *Gonyostomum Semen*, may provide further evidence in favor of Adolph's (1931) contention that the frequency of division is inversely proportional to protoplasmic growth; in other words, the larger the cells in the population, the smaller the frequency of division.

Encystment

Encysted cells appeared in two-day-old hanging-drop cultures of swamp water and also in tightly stoppered swamp-water cultures placed for several days in the dark. In the latter cultures, the encysted material was abundant and lay in yellowish palmelloid masses in the bottom of the liquid. Cultural conditions in these stoppered dishes were obviously unfavorable, for the cysts here exhibited signs of deterioration—the production of much oil and evident disintegration of the chromatophores.

In the hanging-drop cultures, young cysts appeared as spherical bodies 25–36 μ in diameter and surrounded by a thin hyaline sheath (Fig. 17). In some specimens the caudus could be distinguished as an actual projection of the spherical body until the sheath had grown to several microns in thickness. The older cysts (Fig. 18) have a thick gelatinous sheath like that which Cienkowsky (1870) has described and figured for *Vacuolaria virescens*. No trichocyst-like bodies were apparent in these older cysts. Beneath the sheath, the peripheral layer of chromatophores, many oil globules, and several (1–3 in our observations) contractile vacuoles were distinguished. These vacuoles, each measuring 3–4 μ in the diastole, were observed, especially in the young cysts, to discharge directly through the cell membrane. The nucleus was not identified. In old cysts, large clumps of dark brown material appeared in the cytoplasm. The sheath stains intensively with ruthenium red and other dyes, a characteristic which leads us to suspect that pectic substances are present. The sheath obviously prevents the ready passage of dissolved dyes (and other substances?) into the included protoplast.

Division probably occurs in these encysted cells. Our limited time did not permit such observations on this material.

Summary

1. The chloromonad, *Gonyostomum Semen* (Ehrenb.) Diesing, was collected in a sphagnum swamp at Woods Hole, Massachusetts, during July, 1934. The organism has been seen so rarely since it was first described that certain morphological features of the vegetative cells have been imperfectly known, and cell division and encystment have not previously been recorded.

2. The vegetative cells are flattened dorsoventrally and vary in shape in ventral view from lanceolate to circular. The anterior end is two-lobed, and a short caudus terminates the posterior end. No cell wall is present: the cells are somewhat metabolic and under certain conditions become amœboid. The extreme size measurements of a hundred cells were 36–92 $\mu \times$ 23–69 μ. On the ventral surface, a shallow longitudinal groove runs from anterior to posterior regions. Two flagella are present, one projected forward, and one trailing close to the cell body in the ventral groove. A broadly conical cavity lies in the anterior cytoplasm and opens to the outside by a small aperture between the anterior lobes. A large contractile vacuole is seen near this cavity, and several smaller ones may be present. The nucleus is ovoid or subspherical and usually located centrally in the cell. Its structure has not yet been clearly shown. The chromatophores are small, ovoid, and arranged peripherally in the cytoplasm. Oil is the product of metabolism, and under obviously unfavorable conditions it is formed in large quantities. Many hyaline rod-like bodies are arranged radially in a peripheral layer in the cytoplasm. These bodies absorb large amounts of stains from very dilute solutions. Under pressure, or upon addition of chemicals, these bodies shoot out of the cell and usually elongate into threads of slime. They act like the trichocysts in *Paramœcium* and force the worker to exercise extreme care in fixing, preserving, and culturing the organisms.

3. Division of vegetative cells is longitudinal; it occurs, as a rule, only among the smaller cells of the population. Nuclear division and cytoplasmic constriction are described.

4. Encystment of cells was observed in old cultures. Division of encysted cells was not observed.

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